



(11)

EP 0 862 369 B2

(12)

NEW EUROPEAN PATENT SPECIFICATION

After opposition procedure

(45) Date of publication and mention
of the opposition decision:
30.09.2009 Bulletin 2009/40

(45) Mention of the grant of the patent:
07.03.2001 Bulletin 2001/10

(21) Application number: **96939055.8**

(22) Date of filing: **12.11.1996**

(51) Int Cl.:
A23D 9/00 ^(2006.01) **A23D 7/00** ^(2006.01)
C11B 5/00 ^(2006.01) **C11C 1/04** ^(2006.01)
C11C 3/08 ^(2006.01) **C12P 7/64** ^(2006.01)

(86) International application number:
PCT/EP1996/005025

(87) International publication number:
WO 1997/019601 (05.06.1997 Gazette 1997/24)

(54) **COMPOSITION BASED ON FISH OIL**

ZUSAMMENSETZUNG AUF BASIS VON FISCHÖL

COMPOSITION A BASE D'HUILES DE POISSON

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI NL PT SE

(30) Priority: **24.11.1995 EP 95308456**

(43) Date of publication of application:
09.09.1998 Bulletin 1998/37

(73) Proprietors:
• **Unilever N.V.**
3013 AL Rotterdam (NL)
• **Unilever PLC**
London
Greater London EC4P 4BQ (GB)

(72) Inventors:
• **CAIN, Frederick William**
NL-1521 AZ Wormerveer (NL)
• **MOORE, Stephen Raymond**
Sharnbrook,
Bedford MK44 1LQ (GB)
• **McNEILL, Gerald Patrick**
Sharnbrook,
Bedford MK44 1LQ LQ (GB)

(74) Representative: **Joppe, Hermina Laura Petronella**
et al
Unilever Patent Group
Olivier van Noortlaan 120
3133 AT Vlaardingen (NL)

(56) References cited:
WO-A-96/37586 **WO-A-96/37587**

- **JOURNAL OF THE JAPAN OIL CHEMISTS' SOCIETY**, vol. 43, no. 1, 1994, JP, pages 39-43, XP002025976 YUKIHISA TANAKA ET AL.: "SYNTHESIS OF DHA-ENRICHED TRIACYLGLYCEROL"
- **JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY**, vol. 69, no. 11, 1 November 1992, pages 1104-1107, XP000324894 TSUNEO YAMANE ET AL.: "PRODUCTION OF N-3 POLYUNSATURATED FATTY ACID-ENRICHED FISH OIL BY LIPASE-CATALYZED ACIDOLYSIS WITHOUT SOLVENT"
- **PATENT ABSTRACTS OF JAPAN** vol. 017, no. 429 (C-1095), 10 August 1993 & JP 05 095792 A (AGENCY OF IND SCIENCE & TECHNOL; OTHERS: 01), 20 April 1993,
- **DATABASE WPI Section Ch, Week 9517 Derwent Publications Ltd., London, GB; Class B05, AN 95-127359 XP002025981 & JP 07 051 075 A (NIPPON OILS & FATS CO LTD) , 28 February 1995**
- **JOURNAL OF THE JAPAN OIL CHEMISTS' SOCIETY**, vol. 42, no. 1, 1993, JP, pages 30-35, XP002025977 TAKASHI TOYOSHIMA ET AL.: "PREPARATION OF POLYUNSATURATED TRIACYLGLYCEROLS VIA TRANSESTERIFICATION CATALYZED BY IMMOBILIZED LIPASE"
- **INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY**, vol. 27, no. 1, 1992, pages 73-76, XP002025978 E. LIE ET AL.: "ESTERIFICATION OF POLYUNSATURATED FATTY ACIDS WITH LIPASES FROM DIFFERENT SOURCES"
- **DATABASE WPI Section Ch, Week 9550 Derwent Publications Ltd., London, GB; Class B05, AN 95-390578 XP002025982 & JP 07 268 382 A (TAMA SEIKAGAKU KK) , 17 October 1995**

EP 0 862 369 B2

- | | |
|--|--|
| <ul style="list-style-type: none">• JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 71, no. 9, 1994, CHAMPAIGN US, pages 951-954, XP002025979 YUJI SHIMADA ET AL.: "ENRICHMENT OF POLYUNSATURATED FATTY ACIDS WITH GEOTRICHUM CANDIDUM LIPASE"• JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 71, no. 3, 1994, CHAMPAIGN US, pages 331-334, XP002025980 YUKISHA TANAKA ET AL.: "SYNTHESIS OF DOCOSAHEXAENOIC ACID-RICH TRIGLYCERIDE WITH IMMOBILIZED CHROMOBACTERIUM VISCOSUM LIPASE" | <ul style="list-style-type: none">• JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 69, no. 12, 1 December 1992, pages 1210-1214, XP000330808 YUKIHISA TANAKA ET AL.: "CONCENTRATION OF DOCOSAHEXAENOIC ACID IN GLYCERIDE BY HYDROLYSIS OF FISH OIL WITH CANDIDA CYLINDRACEA LIPASE" |
|--|--|

Description

[0001] Fat-compositions, based on fish oils are well-known in the prior art. Many of these compositions were made and applied, because of the health benefits. E.g. Wo 90/04012 discloses triglycerides, containing saturated C₈-C₁₀ fatty acid residues in 1.3 and polyunsaturated fatty acid residues in the 2-position. It is stated, that these triglycerides have beneficial nutritional properties. According to Wo 94/00044 unhardened fish oils have significant health benefits. Above known oils however have one main, common disadvantage: i.e. either the total level of polyunsaturated fatty acids (such as: EPA, DHA, DPA, etc.) is rather low (i.e.: below 35 wt % in total), or if this level is above 35 wt % its oxidative stability is low, while it also displayed high off-taste. Although it is known in the prior art that the oxidative stability of a triglyceride, containing polyunsaturated fatty acids can be increased by incorporation of saturated fatty residues into the triglycerides, the levels of saturated fatty acids were rather high in order to achieve acceptable stability.

[0002] We found new triglycerides, which overcome the drawbacks of the compositions of the prior art. These new compositions combine high levels of polyunsaturated long chain fatty acids, with relatively low levels of saturated fatty acid residues, relatively low off-taste and relatively high oxidative stability. Oxidative stability and off-taste can be evaluated by establishing mean smell scores upon evaluation by a taste-panel. Simultaneously a preference of the panellists can be measured for oils according to the invention, compared with comparative oils. Another method is to measure peroxide values of the different oils. Above triglyceride-compositions can be characterized as fish-oil concentrates, comprising glycerides with:

- (i) at least 40 wt %, preferably 40-55 wt %, more preferably 42-52 wt % of w-3 long chain poly-unsaturated fatty acids, comprising at least DHA and EPA (ie: C_{22:6} and C_{20:5} respectively), and preferably also DPA (ie: C_{22:5}).
- (ii) less than 20 wt %, preferably 2-18 wt %, most preferably 5 - 15 wt % of total saturated fatty acid with 14 - 18 C - atoms.
- (iii) less than 15 wt %, preferably < 12 wt % of C_{18:1} - fatty acid.
- (iv) less than 12 wt %, preferably < 7 wt % of C_{16:1}-fatty acid.
- (v) while DHA and EPA are present in a weight-ratio of 0.5 - 3.0, preferably 0.7 - 2.0.

[0003] Preferred fish oil concentrates comprise triglycerides and diglycerides in a weight ratio of tri:di > 3, preferably 3-50, more preferable 10-35. These concentrates are rich in long chain polyunsaturated fatty acids, whereas its oxidative stability, off-taste and peroxide values are the same or even improved compared with comparative compositions. The SAFA-content of these concentrates is lower than could be expected for above properties.

[0004] Although above concentrates could be added perse to foodproducts, it is often better or easier to use a blend with other triglycerides. Therefore our invention also concerns blends of triglycerides comprising:

0.3 - 95 wt% of the concentrate according to claim 1 or 2, and

99.7 - 5 wt% of a complementary fat, having a solid fat index at 10 °C (N₁₀) that is either at least 5 % more, or at least 5 % less than the N₁₀ of the concentrate according to claim 1 or 2.

[0005] The complementary fat often provides structuring properties to the fatblend. The amounts of complementary fat applied can vary from 98-20 wt %, preferably from 95-60 %. In order to provide structuring characteristics it was found, that the solid fat content (NMR-pulse, not stabilized) of the complementary fat should be >15 at 20°C, preferably >20. Very suitable blends are obtained, if the complementary fat is selected from cocoa butter equivalents, cocoa butter, palm oil or fractions thereof, palmkernel oil or fractions thereof, interesterified mixtures of above fats or fractions or hardened components thereof, or from liquid oil, such as sunflower oil, high oleic sunflower oil, fish oil, soybean oil, rapeseed oil, cottonseed oil, safflower oil, high oleic safflower oil, maize oil or MCT oils, hardened liquid oils or fractions thereof or mixtures of one or more of the fats or oils mentioned.

[0006] The composition of the blend should preferably be selected in such a way, that the blend displays a solid fat content (NMR-pulse; not stabilized) of 0 - 85, preferably 10 - 70, most preferably 20 - 60 at 5 °C and < 30, preferably < 20, most preferably < 5 at 35 °C.

[0007] In order to improve the oxygen stability of our triglycerides or blends, containing them, we prefer to add an effective amount of an oxidation stabilizer, selected from the group consisting of: natural or synthetic tocopherols, BHT, BHA, TBHQ, propylgallate, ascorbylester of fatty acids, free radical scavengers, enzymes with anti-oxidant properties.

[0008] Our invention further concerns food-products, comprising a fat phase, such as spreads, margarine, cream alternative, infant food, chocolate, confectionery, bakery products, sauces, ice-creams, ice-cream coatings, cheese, soups, mayonnaise, dressings, enteral or parental products, wherein the fat phase contains a concentrate or a blend according to claims 1 - 8.

[0009] A very efficient way to dose our new triglycerides, is to make capsules from them. These capsules comprise a filling, encapsulated in an edible coating, wherein the filling consists of the concentrate according to claim 1 or 2 or

the blends according to claims 3-8. In this way our triglycerides can also be eaten, without noticing the disadvantageous off-taste of triglycerides, based on fish-oils.

[0010] Our new composition can be made by blending of the individual triglycerides. However, this is not a very economical way. We found a new, more sophisticated process to prepare them, comprising the following steps:

- (i) a refined fish oil is subjected to an enzymic hydrolysis or alcoholysis, preferably, using *Cand. Rugosa* or *Geotrichum Candidum*
- (ii) the product of (i) is subjected to a treatment for the removal of free fatty acids or its alkylesters
- (iii) the product of (ii) is subjected to an enzymic hydrolysis, in particular using a 1,3-selective lipase or a lipase with a specificity for mono-and diglycerides, such as Amono G-lipase
- (iv) the product of (iii) is washed for the removal of glycerol and dried
- (v) whereupon the product of (iv) is re-esterified.

[0011] In a preferred embodiment the hydrolysis process according to step (i) is performed to a hydrolysis rate of 50 - 80 %. The enzymic treatment according to step (iii) is preferably performed till such a level of free fatty acids, which is at least enough to re-esterify all remaining partial glycerides in the reaction mixture.

[0012] Step (v) of above process can be performed as a directed or non-directed enzymic esterification, or as a directed or non-directed chemical esterification, using a base (in particular Na-methanolate) as a catalyst. Directed chemical esterification is performed by removing the triglycerides formed during the conversion, which are insoluble in the reaction solution.

[0013] In the most preferred embodiment above process is performed on a fish-oil, having following characteristics:

- (a) 10 - 35 wt % of w-3 long chain, polyunsaturated fatty acids, preferably being DHA, EPA and DPA.
- (b) 15 - 35 wt % of saturated fatty acids with 14 - 18 C-atoms.
- (c) 10 - 15 wt % of C_{18:1} - fatty acid.
- (d) 7 - 15 wt % of C_{16:1} - fatty acid.
- (e) DHA : EPA - ratio of 0.5 - 1.5.

EXAMPLES

Example 1:

[0014] 20kg of refined fish oil, with a fatty acid composition shown in table 1.1, was mixed with 200ppm of TBHQ antioxidant and 10kg of 50mM phosphate buffer pH 7. A suspension of 8g of *Candida rugosa* lipase in a small quantity of buffer was then added and the mixture stirred vigorously under a nitrogen atmosphere at 25°C. After 26 hours the free fatty acid (FFA) level in the reaction mixture reached 60%. The oil was heated to 90°C for 10 minutes and the aqueous phase was allowed to settle and drained off. The oil was washed twice with 10kg degassed/demineralised water and dried at 100°C under vacuum. The FFA was separated from the partial glycerides by molecular distillation and the fatty acid composition of each fraction is shown in table 1.1. The corresponding glyceride and FFA compositions are shown in table 1.2. 7.7kg of the partial glyceride fraction obtained as described above was bleached by mixing at 105°C, under vacuum, with 4% of bleaching earth and 0.08% citric acid for 30 minutes followed by filtration. To this oil was added 200ppm TBHQ antioxidant and an equal amount of degassed/demineralised water followed by 3% immobilised *Rhizomucor miehei* lipase based on the weight of oil. Hydrolysis was carried out by stirring for 3.5 hours at 35°C. The lipase was inactivated by heating the reaction mixture to 90°C for 15 minutes and the aqueous phase was allowed to settle and drained off with inactivated lipase. i The partially hydrolysed oil was washed twice with distilled water and after draining the aqueous phases was dried under vacuum at 100°C for 0.5 hours. The composition of the resulting oil is shown in table 1.2.

[0015] After cooling to 55°C, 5% immobilised *Rhizomucor miehei* lipase based on the weight of oil was added and a vacuum of ca. 50mbar was applied. After 24 hours the diglyceride level had dropped to 2.9% and the reaction was stopped by removing the lipase by filtration. The oil was refined and the fatty acid and glyceride composition of the product is given in table 1.1 and table 1.2 respectively.

Table 1.1

Fatty acid compositions (wt%).									
	C14:0	C16:0	C16:1	C16:u	C18:0	C18:1	C18:2	C18:3	C18:4
Fish oil	6.9	18.3	7.9	5.9	3.8	13.6	1.5	0.8	2.1

(continued)

Fatty acid compositions (wt%).									
	C14:0	C16:0	C16:1	C16:u	C18:0	C18:1	C18:2	C18:3	C18:4
Glyceride fraction	4.1	5.9	4.5	6.4	2.5	10.1	1.1	0.6	2.9
FFA fraction	8.9	26.1	10.1	6.0	4.6	16.8	2.0	1.0	1.6
Product	5.3	6.1	5.0	9.3	2.3	9.2	1.5	0.6	3.6
	C20:0	C20:1	C20:5	C20:u	C22:0	C22:1	C22:5	C22:6	C22:u
Fish oil	0.2	1.6	15.9	2.3	0	1.3	2.4	12.3	1.4
Glyceride fraction	0.3	1.8	20.2	3.4	0	2.0	4.5	25.7	2.5
FFA fraction	0.2	1.3	12.3	2.0	0	0.9	0.9	2.4	1.1
Product	0.2	1.4	21.9	2.6	0.2	1.7	3.9	21.9	1.6

Table 1.2

Glyceride and FFA content (wt%).				
	Triglyceride	Diglyceride	Monoglyceride	FFA
Glyceride fraction	72.6	24.6	1.3	1.5
Partially hydrolysed glyceride fraction	43.6	25.1	4.8	26.5
Product	96.8	2.9	0.1	0.1

Example 2:

[0016] A refined fish oil concentrate was made from refined fish oil according to the process described in example 1. The fatty acid composition of the original fish oil and the concentrate is shown in table 2.1. A 10g sample of each was placed in glass bottles with free exposure to air. The bottles were stored at 50°C for 1 week, 40°C for 2 weeks, 20°C for 3 weeks, 5°C for 4 weeks. A group of 6 trained panellists evaluated the quality of the samples by smelling the oil at time 0 and after the storage period. Each panellist assigned a score to the sample on a scale of 0 to 6. A score of 0 corresponds to no detectable odour while a score of 6 is an extremely strong odour. The quality of the samples was determined at time 0 and at the end of the storage period. The mean value of the scores of all the panellists is shown in table 2.2. Table 2.3 shows the preferences of the individual panellists for each sample i.e. the number of people who exhibited a preference for one sample over the other, or who found the samples to have the same odour. The samples were also analyzed for peroxide value (PV) as a measure of oxidative deterioration, a higher PV indicating a greater degree of oxidation. The measured PVs at time 0 and after the storage period are shown in table 2.4.

Table 2.1: Fatty acid composition of the original fish oil and the concentrate (wt%)

	C14 : 0	C16 : 0	C16 : 1	C16 : u	C18 : 0	C18 : 1	C18 : 2	C18 : 3	C18 : 4	C20 : 0	C20 : 1	C20 : 4	C20 : 5	C20 : u	C22 : 0	C22 : 1	C22 : 5	C22 : 6	C22 : u
Fish oil	6.4	15.6	8.0	5.9	3.4	15.1	1.4	1.0	2.2	0.2	1.5	1.2	16.2	1.1	0.1	1.1	2.5	13.2	1.9
Concen trate	3.9	5.5	4.8	6.1	1.9	9.8	0.9	0.5	3.0	1.3	1.3	1.9	17.4	1.6	0.3	1.2	4.5	29.4	2.8
	epa + dpa + dha		C14:0+16 : 0+18:0		C18:1		C16:1		dha:epa										
Fish oil	31.9		25.4		15.1		8.0		0.8										
Concen trate	51.3		11.3		9.8		4.8		1.7										

Table 2.2:

Mean smell scores for fish oil and concentrate at time 0 and after storage at different temperatures for different time periods			
		Mean smell score	
Temperature (°C)	Storage time (weeks)	Fish oil	Concentrate
	0	0.9	1
50	1	2.9	2.9
40	2	2.8	3.4
20	3	2.7	2.7
5	4	2.3	2.1

Table 2.3:

Preferences of individual panellists for fish oil, concentrate or no preference at time 0 and after storage at different temperatures for different time periods				
		No. of panellists who exhibited a preference		
Temperature (°C)	Storage time (weeks)	for fish oil	none	for concentrate
	0	1	4	1
50	1	2	2	2
40	2	4	1	0
20	3	0	4	1
5	4	1	1	1

Table 2.4:

Peroxide values (PV) for fish oil and concentrate at time 0 and after storage at different temperatures for different time periods			
		Peroxide Value (PV)	
Temperature (°C)	Storage time (weeks)	Fish oil	Concentrate
	0	1.8	0.8
50	1	13.3	8.2
40	2	12.2	7.6
20	3	8.5	2.4
5	4	3	1.2

Example 3:

[0017] A partial glyceride concentrate was prepared from refined fish oil by treatment with *C. rugosa* lipase according to the process described in example 1. This partial glyceride material was partially hydrolysed using SP392 lipase also as described in example 1. A portion of this material was then reesterified according to the process in example 1. The fatty acid composition and FFA content of the partially hydrolysed material is shown in table 3.1. A 10g sample of each was placed in glass bottles with free exposure to air. The bottles were stored at 50°C for 1 week, 40°C for 2 weeks, 20°C for 3 weeks, 5°C for 4 weeks. A group of 6 trained panellists evaluated the quality of the samples by smelling the oil at time 0 and after the storage period. Each panellist assigned a score to the sample on a scale of 0 to 6. A score of

EP 0 862 369 B2

0 corresponds to no detectable odour while a score of 6 is an extremely strong odour. The quality of the samples was determined at time 0 and at the end of the storage period. The mean value of the scores of all the panellists is shown in table 3.2. Table 3.3 shows the preferences of the individual panellists for each sample i.e. the number of people who exhibited a preference for one sample over the other, or who found the samples to have the same odour. The samples were also analyzed for peroxide value (PV) as a measure of oxidative deterioration, a higher PV indicating a greater degree of oxidation. The measured PVs at time 0 and after the storage period are shown in table 3.4.

5

10

15

20

25

30

35

40

45

50

55

	C14 : 0	C16 : 0	C16 : 1	C16 : 6 :u	C18 : 0	C18 : 1	C18 : 2	C18 : 3	C18 : 4	C20 : 0	C20 : 1	C20 : 4	C20 : 5	C20 : u	C22 : 0	C22 : 1	C22 : 5	C22 : 6	C22 : u
Hydrolysed	3.8	5.6	4.5	6.6	1.9	9.7	1.0	0.6	3.1	1.6	1.5	1.9	17.2	1.1	0.2	1.2	4.5	30.1	2.4
Reesteri fied	3.7	5.7	4.5	6.4	1.9	9.8	1.1	0.6	3.0	1.6	1.3	1.9	17.1	1.2	0.3	1.3	4.5	30.2	2.5
	epa + dpa + dha		C14:0+16 : 0+18:0		C18:1		C16:1		dha:epa										
Hydrolysed	51.8		11.3		9.7		4.5		1.8										
Reesteri fied	51.8		11.3		9.8		4.5		1.8										
	FFA content																		
Hydrolysed	20.0																		
Reesteri fied	3.9																		

Table 3.2:

mean smell scores for partially hydrolysed concentrate and reesterified concentrate at time 0 and after storage at different temperatures for different time periods

		Mean smell score	
Temperature (°C)	Storage time (weeks)	Hydrolysed	Reesterified
	0	4.4	4.5
50	1	4.7	4.9
40	2	4.7	4.8
20	3	5.8	4.9
5	4	5.0	4.6

Table 3.3: preferences of individual panellists for partially hydrolysed concentrate, reesterified concentrate or no preference at time 0 and after storage at different temperatures for different time periods

		No. of panellists who exhibited a preference		
Temperature (°C)	Storage time (weeks)	for hydrolysed	none	for reesterified
	0	2	3	1
50	1	1	4	1
40	2	2	3	0
20	3	0	1	5
5	4	0	1	4

Table 3.4:

Peroxide values (PV) for partially hydrolysed concentrate and reesterified concentrate at time 0 and after storage at different temperatures for different time periods

		Peroxide Value (PV)	
Temperature (°C)	Storage time (weeks)	Hydrolysed	Reesterified
	0	2.9	2.1
50	1	1.5	4.5
40	2	1.4	5.1
20	3	5.6	5.6
5	4	5.9	3.7

Example 4:

[0018] A refined fish oil concentrate was prepared from refined fish oil according to the process described in example 1. A high saturates concentrate was prepared by dissolving 2 parts of Dynasan (fully hardened soybean oil) in 9 parts of the fish oil concentrate at 80°C for 15 minutes. The concentrate without Dynasan was also heated at 80°C for 15 minutes. Upon cooling to room temperature, the concentrate was liquid and completely clear but the high saturates concentrate formed an opaque solid. The fatty acid composition of the concentrate and the high saturates concentrate is shown in table 4.1. A 10g sample of each was placed in glass bottles with free exposure to air. The bottles were stored at 50°C for 1 week, 40°C for 2 weeks, 20°C for 3 weeks, 5°C for 4 weeks. A group of 6 trained panellists evaluated the quality of the samples by smelling the oil at time 0 and after the storage period. Each panellist assigned a score to the

sample on a scale of 0 to 6. A score of 0 corresponds to no detectable odour while a score of 6 is an extremely strong odour. The quality of the samples was determined at time 0 and at the end of the storage period. The mean value of the scores of all the panellists is shown in table 4.2. Table 4.3 shows the preferences of the individual panellists for each sample i.e. the number of people who exhibited a preference for one sample over the other, or who found the samples to have the same odour. The samples were also analyzed for peroxide value (PV) as a measure of oxidative deterioration, a higher PV indicating a greater degree of oxidation. The measured PVs at time 0 and after the storage period are shown in table 4.4.

5
10
15
20
25
30
35
40
45
50
55

55 50 45 40 35 30 25 20 15 10 5

Table 4.1: Fatty acid composition of the concentrate and the high saturates concentrate (wt%)

	C14 : 0	C16 : 0	C16 : 1	C16 : u	C18 : 0	C18 : 1	C18 : 2	C18 : 3	C18 : 4	C20 : 0	C20 : 1	C20 : 4	C20 : 5	C20 : u	C22 : 0	C22 : 1	C22 : 5	C22 : 6	C22 : u
Concen trate	3.9	5.5	4.8	6.1	1.9	9.8	0.9	0.5	3.0	1.3	1.3	1.9	17.4	1.6	0.3	1.2	4.5	29.4	1.9
High Sats	3.4	4.7	4.4	4.6	21.4	8.1	0.8	0.4	2.4	0.2	1.2	1.7	14.0	0.6	0.0	1.3	3.5	23.3	2.0
	epa + dpa + dha		C14:0+16: 0+18:0		C18:1		C16:1		dha:epa										
Concen trate	51.3		11.3		9.8		4.8		1.7										
High Sats	40.8		29.5		8.1		4.4		1.7										

Table 4.2:

Mean smell scores for concentrate and high saturates concentrate at time 0 and after storage at different temperatures for different time periods			
		Mean smell score	
Temperature (°C)	Storage time (weeks)	Concentrate	High saturates concentrate
	0	1.6	1.6
50	1	2.9	2.5
40	2	2.9	2.9
20	3	2.7	2.9
5	4	2.3	2.3

Table 4.3: Preferences of individual panellists for concentrate and high saturated concentrate at time 0 and after storage at different temperatures for different time periods

		No. of panellists who exhibited a preference		
Temperature (°C)	Storage time (weeks)	for concentrate	none	for high saturates concentrate
	0	1	2	3
50	1	1	1	4
40	2	1	3	1
20	3	1	4	0
5	4	1	3	1

Table 4.4:

Peroxide values (PV) for concentrate and high saturates concentrate at time 0 and after storage at different temperatures for different time periods			
		Peroxide Value (PV)	
Temperature (°C)	Storage time (weeks)	Concentrate	High saturates concentrate
	0	1.1	1.2
50	1	8.6	3.9
40	2	9.9	4.3
20	3	3	3.2
5	4	1.5	1.9

Claims

1. Fish-oil concentrate, comprising glycerides with:

- (i) at least 40 wt %, preferably 40-55 wt %, most preferably 42-52 wt % of w-3 long chain poly-unsaturated fatty acids, comprising at least DHA and EPA (ie: C_{22:6} and C_{20:5} respectively), and preferably also DPA (ie: C_{22:5}).
- (ii) less than 20 wt %, preferably 2-18 wt %, most preferably 5 - 15 wt % of total saturated fatty acid with 14 - 18 C - atoms.
- (iii) less than 15 wt %, preferably < 12 wt % of C_{18:1}-fatty acid.

- (iv) less than 12 wt %, preferably < 7 wt % of C_{16:1}-fatty acid.
- (v) while DHA and EPA are present in a weight-ratio of 0.5 - 3.0, preferably 0.7 - 2.0.
- (vi) and wherein the weight ratio triglycerides to diglycerides is 3-50.

2. Fish oil concentrate, according to claim 1, wherein the concentrate comprises triglycerides and diglycerides in a weight-ratio of 10-35.

3. Blends of triglycerides comprising:

0.3 - 95 wt% of the concentrate according to claim 1 or 2, and
99.7 - 5 wt% of a complementary fat, having a solid fat index at 10 EC (N₁₀) that is either at least 5 % more, or at least 5 % less than the N₁₀ of the concentrate according to claim 1 or 2.

4. Blends of triglycerides, according to claim 3, comprising 2 - 80 wt %, in particular 5 -40 wt % of the concentrate according to claim 1 or 2, and 98 - 20 wt %, in particular 95 - 60 wt % of the complementary fat.

5. Blends according to claims 3 - 4, wherein the complementary fat has a solid fat content (NMR-pulse; not stabilized) of more than 15 at 20 °C, preferably more than 20.

6. Blends according to claims 3 - 5, wherein the complementary fat is selected from cocoa butter equivalents, cocoa butter, palm oil or fractions thereof, palmkernel oil or fractions thereof, interesterified mixtures of above fats or fractions or hardened components thereof, or from liquid oil, such as sunflower oil, high oleic sunflower oil, fish oil, soybean oil, rapeseed oil, cottonseed oil, safflower oil, high oleic safflower oil, maize oil or MCT oils, hardened liquid oils or fractions thereof or mixtures of one or more of the fats or oils mentioned.

7. Blends according to claims 3 - 6, wherein the blend displays a solid fat content (NMR-pulse; not stabilized) of 0 - 85, preferably 10 - 70, most preferably 20 - 60 at 5 °C and < 30, preferably < 20, most preferably < 5 at 35 °C.

8. Triglyceride compositions or blends containing them, according to claims 1 - 7, wherein the compositions or the blends contain an effective amount of an oxidation stabilizer, selected from the group consisting of: natural or synthetic tocopherols, propylgallate, TBHQ, BHT, BHA, free radical scavengers, enzymes with anti-oxidant properties, and ascorbylestere esters of fatty acids.

9. Food products, comprising a fat phase, such as spreads, margarine, cream alternative, infant food, chocolate, confectionery, bakery products, sauces, ice-creams, ice-cream coatings, cheese, soups, mayonnaise, dressings, enteral or parental products, wherein the fat phase contains a concentrate or a blend according to claims 1 - 8.

10. Capsules comprise a filling, encapsulated in an edible coating, wherein the filling consists of the concentrate according to claim 1 or 2 or the blends according to claims 3-8.

11. Process for the preparation of a fish oil concentrate with the composition of claims 1 or 2, wherein

- (i) a refined fish oil is subjected to an enzymic hydrolysis or alcoholysis, preferably using *Candida rugosa* or *Geotrichum Candidum*
- (ii) the product of (i) is subjected to a treatment for the removal of free fatty acids or its alkylesters
- (iii) the product of (ii) is subjected to an enzymic hydrolysis, in particular using a 1.3-selective lipase or a lipase with a specificity for mono- and diglycerides
- (iv) the product of (iii) is washed for the removal of glycerol and dried
- (v) whereupon the product of (iv) is re-esterified.

12. Process according to claim 11, wherein the hydrolysis according to step (i) is performed to a hydrolysis rate of 50 - 80 %.

13. Process according to claim 11, wherein the enzymic hydrolysis according to step (iii) is performed till such level of free fatty acids, which is at least enough to re-esterify all remaining partial glycerides in the reaction-mixture.

14. Process according to claim 11, wherein step (v) is performed as a directed or non-directed enzymic esterification.

15. Process according to claim 11, wherein step (v) is performed as a directed or as a non-directed chemical esterification.

16. Process according to claims 11 - 15, wherein in step (i) of claim 11 a fish oil is applied, having:

- (a) 10 - 35 wt % of w-3 long chain, polyunsaturated fatty acids, preferably being DHA, EPA and DPA.
- (b) 15 - 35 wt % of saturated fatty acids with 14 - 18 C-atoms.
- (c) 10 - 15 wt % of C_{18:1} - fatty acid.
- (d) 7 - 15 wt % of C_{16:1} - fatty acid.
- (e) DHA : EPA - ratio of 0.5 - 1.5.

Patentansprüche

1. Fischölkonzentrat, umfassend Glyceride mit:

- (i) mindestens 40 Gew.-%, bevorzugt 40 bis 55 Gew.-%, insbesondere bevorzugt 42 bis 52 Gew.-%, von w-3-langkettigen mehrfach ungesättigten Fettsäuren, umfassend mindestens DHA und EPA (d.h.: C_{22:6} bzw C_{20:5}) und bevorzugt auch DPA (d.h.: C_{22:5});
- (ii) weniger als 20 Gew.-%, bevorzugt 2 bis 18 Gew.-%, insbesondere bevorzugt 5 bis 15 Gew.-%, der gesamten gesättigten Fettsäure mit 14 bis 18 C-Atomen;
- (iii) weniger als 15 Gew.-%, bevorzugt < 12 Gew.-%, von C_{18:1}-Fettsäure;
- (iv) weniger als 12 Gew.-%, bevorzugt < 7 Gew.-% von C_{16:1}-Fettsäure;
- (v) wobei DHA und EPA in einem Gewichtsverhältnis von 0,5 bis 3,0, bevorzugt 0,7 bis 2,0, vorliegen;
- (vi) und worin das Gewichtsverhältnis Triglyceride zu Diglyceride 3 bis 50 ist.

2. Fischölkonzentrat nach Anspruch 1, worin das Konzentrat Triglyceride und Diglyceride in einem Gewichtsverhältnis von 10 bis 35 umfaßt.

3. Mischungen von Triglyceriden, umfassend:

- 0,3 bis 95 Gew.-% des Konzentrats nach Anspruch 1 oder 2 und
- 99,7 bis 5 Gew.-% eines komplementären Fetts mit einem Festfettindex bei 10 EC (N₁₀), der entweder mindestens 5 % mehr oder mindestens 5 % weniger als der N₁₀ des Konzentrats nach Anspruch 1 oder 2 beträgt.

4. Mischungen von Triglyceriden nach Anspruch 3, umfassend 2 bis 80 Gew.-%, insbesondere 5 bis 40 Gew.-%, des Konzentrats nach Anspruch 1 oder 2, und 98 bis 20 Gew.-%, insbesondere 95 bis 60 Gew.-%, des komplementären Fetts.

5. Mischungen nach den Ansprüchen 3 bis 4, worin das komplementäre Fett einen Festfettgehalt (NMR-Puls; nicht stabilisiert) bei 20°C von mehr als 15, bevorzugt mehr als 20 aufweist.

6. Mischungen nach den Ansprüchen 3 bis 5, worin das komplementäre Fett ausgewählt ist aus Kakaobutter-Äquivalenten, Kakaobutter, Palmöl oder Fraktionen hiervon, Palmkernöl oder Fraktionen hiervon, umgeesterten Mischungen obiger Fette oder Fraktionen oder gehärteter Komponenten hiervon, oder von flüssigem Öl, wie Sonnenblumenöl, oleinreichem Sonnenblumenöl, Fischöl, Sojabohnenöl, Rapsöl, Baumwollöl, Saffloröl, oleinreichem Saffloröl, Maisöl oder MCT-Ölen, gehärteten flüssigen Ölen oder Fraktionen hiervon, oder Mischungen eines oder mehrerer der erwähnten Fette oder Öle.

7. Mischungen nach den Ansprüchen 3 bis 6, worin die Mischung einen Festfettgehalt (NMR-Puls; nicht stabilisiert) bei 5°C von 0 bis 85, bevorzugt 10 bis 70, insbesondere bevorzugt 20 bis 60, und bei 35°C < 30, bevorzugt < 20, insbesondere bevorzugt < 5, zeigt.

8. Triglyceridzusammensetzungen oder diese enthaltende Mischungen nach den Ansprüchen 1 bis 7, worin die Zusammensetzungen oder Mischungen eine wirksame Menge eines Oxidationsstabilisators enthalten, ausgewählt aus der aus natürlichen oder synthetischen Tocopherolen, Propylgallat, TBHQ, BHT, BHA, Radikalfängern, Enzymen mit Antioxidanz-Eigenschaften und Ascorbylestern von Fettsäuren bestehenden Gruppe.

9. Nahrungsmittelprodukte, umfassend eine Fettphase, wie Aufstriche, Margarine. Rahmersatz, Babynahrung, Scho-

kolade, Konfekt, Backprodukte, Soßen, Eiscremen, Eiscreme-Überzüge, Käse, Suppen, Mayonnaise, Dressings, enterale oder parenterale Produkte, worin die Fettphase ein Konzentrat oder eine Mischung nach den Ansprüchen 1 bis 8 enthält.

- 5 10. Kapseln, umfassend eine Füllung, eingekapselt in eine essbare Beschichtung, worin die Füllung aus dem Konzentrat nach dem Anspruch 1 oder 2 oder aus den Mischungen nach den Ansprüchen 3 bis 8 besteht.
11. Verfahren zur Herstellung eines Fischölkonzentrats mit der Zusammensetzung nach den Ansprüchen 1 oder 2, worin
 - 10 (i) ein raffiniertes Fischöl einer enzymatischen Hydrolyse oder Alkoholyse, bevorzugt unter Verwendung von *Cand. Rugosa* oder *Geotrichum Candidum*, unterzogen wird;
 - (ii) das Produkt von (i) einer Behandlung zum Entfernen der freien Fettsäuren oder ihrer Alkylester unterzogen wird;
 - 15 (iii) das Produkt von (ii) einer enzymatischen Hydrolyse, insbesondere unter Verwendung einer 1,3-selektiven Lipase oder einer Lipase mit einer Spezifität für Mono- und Diglyceride, unterzogen wird;
 - (iv) das Produkt von (iii) zur Entfernung von Glycerol gewaschen und getrocknet wird;
 - (v) woraufhin das Produkt von (iv) erneut verestert wird
12. Verfahren nach Anspruch 11, worin die Hydrolyse nach Schritt (i) bis zu einem Hydrolysegrad von 50 bis 80 %
20 durchgeführt wird.
13. Verfahren nach Anspruch 11, worin die enzymatische Hydrolyse nach Schritt (iii) bis zu einem Gehalt an freien Fettsäuren durchgeführt wird, der zumindest ausreicht, alle verbliebenen partiellen Glyceride in der Reaktionsmischung erneut zu verestern.
25
14. Verfahren nach Anspruch 11, worin Schritt (v) als eine gerichtete oder ungerichtete enzymatische Veresterung durchgeführt wird.
15. Verfahren nach Anspruch 11, worin Schritt (v) als eine gerichtete oder ungerichtete chemische Veresterung durchgeführt wird.
30
16. Verfahren nach den Ansprüchen 11 bis 15, worin in Schritt (i) von Anspruch 11 ein Fischöl eingesetzt wird, mit:
 - 35 (a) 10 bis 35 Gew.-% w-3-langkettiger mehrfach ungesättigte Fettsäuren, bevorzugt DHA, EPA und DPA;
 - (b) 15 bis 35 Gew.-% gesättigte Fettsäuren mit 14 bis 18 C-Atomen;
 - (c) 10 bis 15 Gew.-% C_{18:1}-Fettsäure;
 - (d) 7 bis 15 Gew.-% C_{16:1}-Fettsäure;
 - (e) einem DHA:EPA-Verhältnis von 0,5 bis 1,5.

Revendications

1. Concentré d'huile de poisson, comprenant des glycérides, avec :
 - 45 (i) au moins 40 % en masse, de préférence 40 - 55 % en masse, de façon plus préférentielle 42 - 52 % en masse d'acides gras w-3polyinsaturés à chaîne longue, comprenant au moins du DHA et de l'EPA (c'est à dire en C_{22:6} et en C_{20:5} respectivement), et de préférence également du DPA (c'est-à-dire en C_{22:5}).
 - (ii) moins de 20 % en masse, de préférence de 2 à 18 % en masse, de façon plus préférentielle de 5 - 15 % en masse d'acide gras totalement saturés avec 14 - 18 atomes de carbone.
 - 50 (iii) moins de 15 % en masse, de préférence inférieur à 12 % en masse d'acides gras en C_{18:1}.
 - (iv) moins de 12 % en masse, de préférence inférieur à 7 % en masse d'acides gras en C_{16:1}.
 - (v) les DHA et les EPA étant présents dans un rapport en masse compris entre 0,5 et 3,0, de préférence 0,7 - 2,0.
 - (vi) et dans laquelle le rapport en masse des triglycérides aux diglycérides est compris entre 3 et 50.
- 55 2. Concentré d'huile de poisson selon la revendication 1, dans lequel le concentré comprend des triglycérides et des diglycérides dans un rapport en masse de 10 - 35.
3. Mélanges de triglycérides comprenant :

- ▶ 0,3 - 95 % en masse du concentré selon les revendications 1 ou 2 ; et
- ▶ 99,7 - 5 % en masse d'une matière grasse complémentaire, ayant un indice de matière grasse solide à 10 E C (N₁₀) qui est soit d'au moins 5 % supérieur, soit d'au moins 5 % inférieur au N₁₀ du concentré selon la revendication 1 ou 2.

- 5 4. Mélanges de triglycérides selon la revendication 3, comprenant de 2 à 80 % en masse, plus particulièrement de 5 à 40 % en masse du concentré selon la revendication 1 ou 2, et 98 à 20 % en masse, plus particulièrement de 95 à 60% en masse de la matière grasse complémentaire.
- 10 5. Mélanges selon les revendications 3 - 4, dans lesquels la matière grasse complémentaire a une teneur en matière grasse solide (RMN par impulsions ; non stabilisée) supérieure à 15 à 20°C, de préférence supérieure à 20.
- 15 6. Mélanges selon les revendications 3 - 5, dans lesquels la matière grasse complémentaire est sélectionnée à partir des équivalents du beurre de cacao, du beurre de cacao, de l'huile de palme ou des fractions de celle-ci, de l'huile de palmiste ou des fractions de celle-ci, de mélanges interestérifiés des matières grasses énumérées ci-dessus ou des fractions ou des composants durcis de celles-ci, ou à partir d'huile liquide telle que l'huile de tournesol, l'huile de tournesol hautement oléique, l'huile de poisson, l'huile de soja, l'huile de graine de colza, l'huile de graine de coton, l'huile de carthame, l'huile de carthame hautement oléique, l'huile de maïs ou les huiles MCT, les huiles liquides durcies ou des fractions de celles-ci ou des mélanges d'une ou de plusieurs des matières grasses ou des huiles mentionnées.
- 20 7. Mélanges selon les revendications 3 - 6, dans lesquels le mélange présente une teneur en matière grasse solide (RMN par impulsions ; non stabilisée) de 0 - 85, de préférence de 10 - 70, de la façon la plus préférentielle de 20 - 60 à 5°C et inférieure à 30, de préférence inférieure à 20 et de la façon la plus préférentielle inférieure à 5 à 35°C.
- 25 8. Compositions de triglycérides ou mélanges les contenant, selon les revendications 1 - 7, dans lesquels les compositions ou les mélanges les contenant contiennent une quantité efficace d'un stabilisateur d'oxydation sélectionné à partir du groupe composé des tocophérols naturels ou synthétiques, BHT, BHA, TBHQ, propygalates, ester ascorbyles d'acides gras, des fixateurs de radicaux libres, des enzymes ayant des propriétés anti oxydantes.
- 30 9. Produits alimentaires comprenant une phase grasse tels que les pâtes à tartiner, la margarine, les produits de remplacement de la crème, les aliments pour bébés, le chocolat, les confiseries, les produits de boulangerie, les sauces, les glaces, les enrobages de glaces, le fromage, les soupes, la mayonnaise, les vinaigrettes, les produits pour administration entérale ou parentérale, dans lesquels la phase grasse contient un concentré ou un mélange selon les revendications 1 à 8.
- 35 10. Capsules comprenant une garniture enfermée dans un enrobage comestible, dans lesquelles la garniture est composée du concentré selon les revendications 1 ou 2 ou des mélanges selon les revendications 3 à 8.
- 40 11. Procédé de préparation d'un concentré d'huile de poisson à partir de la composition selon les revendications 1 ou 2 et comprenant les étapes consistant à :
 - (i) soumettre une huile de poisson raffinée à une hydrolyse enzymatique ou à une alcoololyse, en utilisant de préférence *Cand Rugosa* ou *Geotrichum Candidum* ;
 - 45 (ii) soumettre le produit obtenu dans le cadre de l'étape (i) à un traitement permettant de retirer les acides gras libres ou ses alkylesters ;
 - (iii) soumettre le produit obtenu dans le cadre de l'étape (ii) à une hydrolyse enzymatique, en particulier en utilisant une lipase 1,3-sélective ou une lipase ayant une spécificité aux mono- et aux diglycérides ;
 - (iv) laver le produit obtenu dans le cadre de l'étape (iii) afin d'en retirer le glycérol, puis le sécher ;
 - 50 (v) et enfin, re-estérifier le produit obtenu dans le cadre de l'étape (iv).
12. Procédé selon la revendication 11, dans lequel l'hydrolyse de l'étape (i) est effectuée à un rythme d'hydrolyse de 50 - 80 %.
- 55 13. Procédé selon la revendication 11, dans lequel l'hydrolyse enzymatique de l'étape (iii) est effectuée jusqu'à un niveau d'acides gras libres qui est au moins suffisant pour re-estérifier tous les glycérides partiels restant dans le mélange de réaction.

14. Procédé selon la revendication 11, dans lequel l'étape (v) est effectuée en tant qu'estérification enzymatique dirigée ou non dirigée.

5 15. Procédé selon la revendication 11, dans lequel l'étape (v) est effectuée en tant qu'estérification chimique dirigée ou non dirigée.

16. Procédé selon les revendications 11 - 15, dans lequel dans l'étape (i) de la revendication 11, on applique une huile de poisson ayant :

10 (a) 10 - 35 % en masse d'acides gras w-3, polyinsaturés et à chaîne longue, de préférence du DHA, de l'EPA et du DPA ;

(b) 15 - 35 % en masse d'acides gras saturés avec de 14 à 18 atomes de carbone ;

(c) 10 - 15 % en masse d'acide gras en C_{18:1} ;

(d) 7 - 15 % en masse d'acide gras en C_{16:1} ;

15 (e) un ratio DHA : EPA de 0,5 - 1,5.

20

25

30

35

40

45

50

55